Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 1-4, 6, 8-11 and 16-21 are pending in the application, with claims 1 and 16 being the independent claims.

Claims 1 and 10 are sought to be amended. The amendment to claim 1 replaces the phrase "directly contacting the nerve cells with . . ." with "administering directly to nerve cells . . ." Support for this amendment can be found throughout the specification, for example, at page 8, lines 12-15. The amendment to claim 10 is a grammatical change, inserting an "a" directly before "neurotrophic factor." No new matter is added by way of these amendments. These amendments are believed to present the claims in condition for allowance or in better form for consideration on appeal. *See* 37 C.F.R. § 1.116(b). It is therefore respectfully requested that the amendments after final Office Action be entered and considered.

Based on the above amendment and the following remarks, Applicants respectfully request that the Examiner reconsider all outstanding rejections and that they be withdrawn.

I. Ninth Supplemental Information Disclosure Statement

Applicants note that a ninth supplemental Information Disclosure Statement, including Form PTO-1449 (8 sheets) and 22 cited documents, was filed in the present application on December 2, 2004. The Examiner has not provided an initialed copy of the

Form PTO-1449 and has not acknowledged that the documents submitted therewith have been considered. Applicants respectfully request that the Examiner initial and return a copy of the Form PTO-1449 and indicate in the official file wrapper of this patent application that the documents have been considered.

II. Examiner Interview

According to an Interview Summary issued on December 14, 2004, a telephonic interview with the Examiner was conducted on December 6, 2004, during which a draft copy of proposed claim amendments was discussed.

III. Nonstatutory Double Patenting Rejections

A. Application No. 09/728,207

Claim 16 was provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-3 of copending Application No. 09/728,207. *See* Office Action, page 3. Applicants respectfully request that this rejection be held in abeyance until allowable subject matter is established. At that stage, Applicants will consider filing a terminal disclaimer over the above-cited patent application.

B. Application No. 09/720,003

Claim 16 was provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-3 of Application No. 09/720,003. *See* Office Action, page 4. Applicants note that Application No. 09/720,003

issued as U.S. Patent No. 6,740,524 on May 25, 2004. *See* Exhibit A (showing the cover page, claims and Certificate of Correction for U.S. Patent No. 6,740,524). Accordingly, this provisional rejection, based on the '003 application, is no longer appropriate. Furthermore, Applicants submit that the Examiner's basis for this rejection is not applicable in view of the claims that issued in U.S. Patent No. 6,740,524. According to the Examiner:

> Claim 16 of the instant application is drawn to any negative strand RNA virus. Claims 1-3 of copending Application No. 09/720,003 are drawn to the subset of negative strand RNA viruses that are sendai viruses comprising insertions, deletions, or gene inactivations that do not remove the disseminative capacity of the virus.

See Office Action, page 4.

Applicants note that issued claim 3 of U.S. Patent No. 6,740,524 depends from claim 2, which depends from claim 1. Accordingly, claim 3 incorporates by reference all of the limitations recited in claims 1 and 2. *See* 35 U.S.C. § 112, fourth paragraph. Claim 3 of U.S. Patent No. 6,740,524 is therefore directed to a vector comprising a DNA fragment encoding a fusion protein comprising (i) a protein having both nuclear translocation and cell adhesion activities and (ii) a protein comprising a lambda phage head protein, wherein said protein having both nuclear translocation and cell adhesion activities comprises the amino acid sequence set forth in SEQ ID NO: 1 and wherein said lambda phage head protein comprises D protein of lambda phage. *See* U.S. Patent No. 6,740,524, column 17, lines 2-11. The Examiner has not explained how such a claim overlaps or renders obvious present

claim 16, directed to a specific negative-sense RNA viral vector, namely a Sendai viral vector. Accordingly, Applicants request reconsideration and withdrawal of this rejection.

C. Application No. 09/720,979

Claims 1-6, 8-11, 15 and 16 were provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-6, 8-10 and 14-18 of copending Application No. 09/720,979. *See* Office Action, page 5. Applicants note that U.S. Application No. 09/720,979 is abandoned. *See* Exhibit B (showing the Notice of Abandonment that was issued in U.S. Application No. 09/720,979 on January 7, 2005). Accordingly, this rejection is moot.

D. Application No. 10/444,661

Claim 16 was provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claim 1 of copending Application No. 10/444,661. *See* Office Action, page 5. Applicants respectfully request that this rejection be held in abeyance until allowable subject matter is established. At that stage, Applicants will consider filing a terminal disclaimer over the above-cited patent application.

IV. Claim Rejections Under 35 U.S.C. § 112, First Paragraph

A. Written Description

Claims 9, 10 and 16 were rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement. *See* Office Action, pages 6-7 and 9. Applicants respectfully traverse this rejection.

The Examiner asserted that the exemplary disclosed species of the recited *Markush* group of fibroblast growth factors, nerve growth factors, apoptosis inhibitors, heat shock proteins, peroxidases and neurotrophic factors "do not contain a common structure from which to distinguish the various members of any of the aforementioned genera." *See* Office Action, page 9. According to the Examiner, "the only other characteristics [of the members of the claimed genus] are the functional characteristic common to all of the genera: capable of protecting the brain from ischemia, as well as being an apoptosis-suppressing gene, a nerve growth factor, a heat shock protein, or a peroxidase." *See* Office Action, pages 9-10. Thus, the Examiner concluded that the information disclosed in the specification "is not deemed sufficient to reasonably convey to one skilled in the art that Applicant is in possession of any apoptosis-inhibitor, any nerve growth factor, any heat shock protein, or any peroxidase, at the time the application was filed." *See* Office Action, page 10.

In response, Applicants note that members of a genus need not necessarily share significant common structure for that genus to be adequately described. Rather, the test for adequate written description is whether the application conveys with reasonable clarity to those skilled in the art that, as of the filing date, the inventor was in possession of the claimed invention, namely in possession of the necessary common features or attributes possessed by members of the genus claimed. In the context of a genus claim, possession can be evidenced through disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics. Moreover, if "the broader concept would naturally occur to one

skilled in the art upon reading [Applicants'] specification," then the genus is adequately described. *See Levi Strauss & Co. v. Golden Trade, S.R.L.*, 1991 WL 710822 (S.D.N.Y. Dec. 1, 1995) (quoting *In re Smythe*, 480 F.2d 1376, 1384 (C.C.P.A. 1973); *Waldemar Link, GmbH & Co. v. Ostoenics Corp.*, 32 F.3d 556, 558 (Fed. Cir. 1994)).

As noted previously, possession of a genus may be satisfied through description of a "representative number of species," e.g., species which are actually described that are representative of the entire genus. While the Examiner cites to case law which suggests that a single species can rarely, if ever, afford sufficient support for a generic claim, the published guidelines for assessing adequacy of written description explicitly state that "a single species may, in some instances, provide an adequate written description of a generic claim when the description of the species would evidence to one of ordinary skill in the art that the invention includes the genus." See FR Vol. 66, No. 4 (2001), p, 1102 - comment (16). What constitutes a representative number is an inverse function of the skill and knowledge in the art. In other words, when there is substantial variation with the genus, one must describe a sufficient variety of species to reflect the variation; however, when the genus lacks variation, a single representative species may suffice. Satisfactory disclosure of a representative number of species depends on whether one of skill in the art would recognize that the applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed.

In this case, the Examiner has based the written description rejection on the ground that "Applicant has not provided common structure to distinguish the various members of the genera from each other." *See* Office Action, page 11. However, since there is a strong

presumption that a specification provides adequate written description of the claimed subject matter, it is the Examiner's burden to affirmatively demonstrate how the specification fails to convey possession of the claimed invention. As the USPTO's training materials on written description clearly indicate, the Examiner has the initial burden of showing that the species encompassed by the claimed genus are expected to be widely divergent and highly variable in terms of structure and the like. *See, e.g.*, Example 17. *See also In re Wertheim*, 541 F.2d 257, 263, 191 USPQ 90, 97 (CCPA 1976); MPEP § 2163.04.

In this case, while each of the recited categories of proteins (fibroblast growth factors, nerve growth factors, apoptosis inhibitors, heat shock proteins, peroxidases and neurotrophic factors) may each arguably represent a separate "genus," Applicants submit that these so-called genera are neither extensive nor substantially variable. Moreover, the members of each "genus" clearly possess a requisite number of common features and attributes such that "the broader concept would naturally occur to one skilled in the art upon reading [Applicants'] specification." See *Levi Strauss & Co. v. Golden Trade, S.R.L.*, 1991 WL 710822 (S.D.N.Y. Dec. 1, 1995) (quoting *In re Smythe*, 480 F.2d 1376, 1384 (C.C.P.A. 1973); *Waldemar Link, GmbH & Co. v. Ostoenics Corp.*, 32 F.3d 556, 558 (Fed. Cir. 1994)). Although some structural variations may exist among the recited categories of protein, given the level of skill and knowledge in the art and the degree of structural and functional conservation associated with each of the recited genera, one of skill in the art would recognize that Applicants were in possession of the necessary common attributes or features possessed by the members of the genera in view of the species disclosed. *See* discussion below for specific examples. Accordingly, given the skill and knowledge in the art, the

examples disclosed in the instant specification constitute a representative number of species sufficient to demonstrate possession of the corresponding genera.

For example, contrary to the Examiner's suggestion, the members of the FGF family share not only common function but also common structure. In fact, at least 19 members of the FGF family by definition share a number of relevant identifying characteristics, including three dimensional structure and sequence homology, physical and/or chemical properties, and functional characteristics. For example, the members of the FGF family are beta proteins having a distinctive beta-trefoil superfold, the only superfold to exhibit 3-fold structural symmetry. Furthermore, they all possess significant conserved sequences and motifs as well as structure which allows them to interact with at least one of four cell-surface receptors, identified as FGFR1 - FGFR4. Finally, as noted previously, like NGFs, they are known to play a role in self-protection against ischemic neuron and, thus, can inhibit post-ischemic neuronal death when administered before or immediately after ischemic modalities of cerebral protection. Thus, given the fact that the FGF family is a well-studied, well-characterized, well-recognized class of proteins, given the skill and knowledge in the art, and given the fact that Applicants explicitly describe three distinct species, namely, FGF-1, FGF-2, and FGF-5, that are representative of the FGF family of growth factors, Applicants submit that the full scope of the subject matter of the present claims would naturally occur to one skilled in the art upon reading the specification and the FGF examples described.

Regarding the supposed "genus" of nerve growth factors, Applicants respectfully submit that the Examiner's statements are in error. While some sequence variation exists across species, contrary to the Examiner's suggestion, NGF does not represent a family of

proteins but rather a singular protein that is a member of the family neurotrophins that induce the survival and proliferation of neurons. NGF is a zinc finger protein that asymmetrically binds as a homodimer to a single p75 receptor. Indeed, the members of the NGF "genus" retain significant conserved sequence, structure, and function. Thus, given the fact that NGF is a well-studied, well-characterized, well-recognized protein, given the skill and knowledge in the art, and given the fact that Applicants explicitly describe human NGF (a representative species of the NGF "genus"), Applicants submit that the full scope of the subject matter of the present claims would naturally occur to one skilled in the art upon reading the specification and the examples described.

Regarding the "genus" of apoptosis inhibitors, contrary to the Examiner's suggestion, members of the inhibitor of apoptosis family (IAPs) share not only common function but also common structure. In terms of function, IAPs bind to caspases, the principle effectors of apoptosis, and inhibit their enzymatic activity. IAPs possess significant conserved sequences and structure which allow them to specifically interact with and inhibit these primary apoptosis enzymes. Thus, given the fact that the IAP family is a well-studied, well-characterized, well-recognized class of proteins, given the skill and knowledge in the art, and given the fact that Applicants explicitly describe three distinct species of caspase inhibitor – namely, Crm-A (an inhibitor of human caspase-1 and granzyme B); ILP (also known as X-linked inhibitors of apoptosis or XIAPs) and bcl-2 (a family of apoptosis suppressors characterized by the ability to bind to and inactivate adaptor proteins required for pro-caspase processing) – that are representative of the IAP family of proteins, Applicants

submit that the full scope of the subject matter of the present claims would naturally occur to one skilled in the art upon reading the specification, including the examples described.

Regarding the "genus" of heat shock proteins, contrary to the Examiner's suggestion, the members of the small heat shock protein family share not only common function but also common structure. Specifically, although the proteins extend over all kingdoms, they are all characterized by a common core domain with variable N- and C-terminal extensions. The relatively hydrophobic N-terminus plays a critical role in promoting and controlling high-order aggregation. While there indeed exists structural variability within the superfamily, we respectfully submit that the examples provided (e.g., ORP-150 or the oxygen-regulated protein 150) sufficiently represent this variation.

Moreover, given the fact that the HSP family is a well-studied, well-characterized, well-recognized class of proteins and given the skill, knowledge and state of the art, Applicants submit that the full scope of the subject matter of the present claims would naturally occur to one skilled in the art upon reading the specification, including the examples described.

Regarding the "genus" of peroxidases, as noted previously, peroxidases have been shown to play a role in protecting tissues from ischemic damage. Peroxidases are defined as enzymes that catalyze the reduction of hydrogen peroxide by a substrate that loses two hydrogen atoms. In the context of the instant invention, the term "peroxidase" includes a family of haem enzymes found primarily in the white blood cells that consist of a protein complexed with hematin groups that catalyzes the oxidation of various substances by peroxides. Such peroxidases are typically compounds of iron complexed in a porphyrin

(tetrapyrrole) ring that differ in side chain compositions; haems are the prosthetic groups of cytochromes and are found in most oxygen carrier compositions. Accordingly, peroxidases indeed share significant structural as well as functional characteristics. Given the fact that the peroxidases comprise a well-studied, well-characterized, well-recognized class of proteins and given the skill, knowledge and state of the art, we submit the broader concept claimed would naturally occur to one skilled in the art upon reading the earlier specification, including the examples described.

Regarding the "genus" of neurotrophic factors, as noted previously, the instant specification describes a number of illustrative neurotrophic factors, including NGF (nerve growth factor), CNTF (ciliary neurotrophic factor), BDNF (brain-derived neurotrophic factor or neurotrophin-2), and GDNF (glial cell-derived neurotrophic factor). *See* specification, page 10, lines 15-20. Other well known examples of neurotrophic factors include members of the neurotrophin family, which includes, in addition to BDNF, neurotrophin-3, neurotrophin-4, and beta-nerve growth factor. Contrary to the Examiner's suggestion, these proteins in fact share structural as well as functional characteristics. Indeed, neurotrophic factors are broadly categorized as cystine knot cytokines, a family of small proteins having a disulphide-rich fold and sharing a common core that is all-beta. *See* the SCOP database at http://scop.berkeley.edu/index.html for additional details. Accordingly, Applicants submit that the claimed "genus" of neurotrophic factors is a well-studied and well-characterized family of proteins that is not substantially variable. Moreover, given the skill and knowledge in the art, the examples disclosed in the instant specification constitute a representative number of species sufficient to demonstrate possession thereof. In other

words, although some structural variations may exist among the species, given the level of skill and knowledge in the art and the degree of structural and functional conservation associated with the genus, one of skill in the art would recognize that Applicants were in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed.

In sum, Applicants submit that the Examiner's characterization of the recited genera is in error and that each genus is supported by an adequate written description, including a sufficient number of representative species. Accordingly, Applicants request reconsideration and withdrawal of the written description rejection.

B. Enablement

Claims 1-5, 8-11 and 16-21 were rejected under 35 U.S.C. § 112, first paragraph, for lack of enablement. *See* Office Action, page 12. Applicants respectfully traverse this rejection.

According to the Examiner, the specification, while being enabling for specific embodiments of the present invention, allegedly "does not reasonably provide enablement for any method of administration, any ex vivo method, or the treatment of any mammal." *See* Office Action, page 12. The Examiner has made several assertions regarding the supposed difficulties relating to *in vivo* foreign gene delivery and expression, particularly as to efficiency of gene transfer and expression (citing Eck, Lamb et al. and Yonemitsu), as well as the supposed inherent difficulties in targeting intended cells and achieving long term

expression of the transgene (citing Deonarian, Verma, Gorecki, and Nakanishi) and the supposed unpredictability of gene therapy extrapolated to human systems (citing Crystal and Gura). *See* Office Action dated May 5, 2004, pages 15-25.

At the outset, Applicants note that the Examiner apparently has regarded the phrase "directly contacting" to be broader than "direct administration." *See* Office Action, page 13. According to the Examiner, "[i]t is noted that Applicant's newly amended claim 1 requires the nerve cells to be contacted directly with the vector, however, such is not equivalent to direct administration, as required by the examiner." *See id.* Although Applicants respectfully disagree, claim 1 has been amended to recite "administering directly to the nerve cells . . ." This amendment has been made solely to expedite prosecution. Applicants believe that the amendment to claim 1 fully accommodates the issue of whether the specification enables "any method of administration," as well as the issue of whether the specification enables "any ex vivo method."

Thus, in view of the amendment to claim 1, Applicants believe that the sole remaining issue regarding enablement relates to whether the specification adequately enables "the treatment of any mammal." Applicants respectfully remind the Examiner that the proper standard for compliance with enablement is not *absolute predictability* but *objective enablement*; evidence need not be *conclusive* but merely *convincing*. Accordingly, Applicants again submit that the compelling animal data presented in the specification is sufficiently convincing that one of ordinary skill in the art would not doubt the feasibility of the claimed invention or its application to mammals other than rodents. Moreover, the *in*

vivo successes documented in the Examples of the instant specification clearly outweigh any speculative allegations of unpredictability asserted by the Examiner.

Contrary to the Examiner's suggestion, the "scaling up" of the disclosed procedures for application to other mammals, including humans, is considered routine experimentation well within the purview of one of ordinary skill. Thus, given the explicit disclosure of specific *in vitro* and *in vivo* working examples, using models that reasonably correlate to mammals other than rodents, Applicants respectfully submit that one skilled in the art would be able to make and use the claimed invention without undue experimentation.

According to the Examiner's apparent view of the enablement requirement, an applicant would have to submit conclusive data from human clinical trials in order to adequately enable an *in vivo* gene therapy method applicable to humans. This is clearly in conflict with the statute, the rules and the guidelines of the MPEP. Specifically, under the current case law, clinical efficacy is not required to show that a therapeutic process is operable. As stated in M.P.E.P. § 2107.01, the "courts have found utility for therapeutic inventions, despite the fact that an applicant is at a very early stage in the development of a therapeutic regimen" or that a therapeutic treatment regimen is not at a stage where it is ready to be practiced on humans. *Cross v. Iizuka*, 753 F.2d 1040, 224 U.S.P.Q. 739 (Fed. Cir. 1985); *In re Brana*, 51 F.3d 1560, 34 U.S.P.Q.2d 1436 (Fed. Cir. 1995). It is not within the province of the USPTO to require proof of efficacy in animals to grant a patent including claims to therapeutic methods. The PTO guidelines, in fact are explicit on this point: "Office personnel should not impose on applicants the unnecessary burden of providing evidence from human clinical trials. There is no decisional law that requires an applicant to

provide data from human clinical trials to establish utility for an invention related to treatment of human disorders." (M.P.E.P. § 2107.03). The guidelines further state that "[t]he Office must confine its review of patent applications to the statutory requirements of the patent law, and in quoting *In re Brana, supra*, that "FDA approval, however, is not a prerequisite for finding a compound useful within the meaning of the patent laws". *Id.* In fact, all that is required by the patent laws is that a "*reasonable correlation*" exist between the scope of the claims and the scope of enablement. Citing to M.P.E.P. § 2164.02, ""correlation" as used herein refers to the relationship between *in vitro* or *in vivo* animal model assays and a disclosed or a claimed method of use." If a particular model is recognized as correlating to a specific condition, then it should be accepted as such unless the Examiner has evidence that the model does not correlate. *In re Brana, supra* at 1566. Since the initial burden is on the Examiner to give reasons for lack of enablement, the Examiner must also give reasons for a conclusion of lack of correlation for an *in vitro* or *in vivo* animal model example. As stated in *Cross v. Iizuka, supra*, at 1050, a rigorous or an invariable exact correlation is not required.

In the instant case, the positive findings associated with the *in vitro* and *in vivo* delivery and expression in specific nerve cells of both reporter genes, such as firefly luciferase and β-galactosidase, and therapeutic genes, such as β-glucuronidase, directly correlate to treatment of gene-based diseases in animals, including humans. Given this correlation, there is no reason to doubt Applicants' assertion that the instant Sendai viral vectors are fully enabled for treating neurodegenerative conditions, such as those associated with Parkinson's disease, ischemia and the like.

Furthermore, Applicants note that gene therapy using viral vectors is not unpredictable, as evidenced by recent reports. *See, e.g.*, J. C. Glorioso *et al.*, *Journal of NeuroVirology*, 9:165-172, (2003), attached hereto as Exhibit C. This report supports a reasonable correlation between *in vitro* or *in vivo* animal model data and human treatment. Although this reference was published after the effective filing date of the present application, its results strongly support Applicants' contention that, at the time of the effective filing date of the present application, a person of ordinary skill in the art would have been able to make and use the subject matter of the present claims without undue experimentation. Accordingly, one skilled in the art would not doubt the reasonable predictability of the field of the invention.

For the reasons given above, Applicants submit that the scope of the present claims is commensurate in scope with the enablement provided in the present specification. Accordingly, Applicants request reconsideration and withdrawal of the enablement rejection in view of the amendments to the claims and the remarks herein.

Conclusion

All of the stated grounds of rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that

personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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Date: APRIL 13, 2005

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09/720,979	03/07/2001	Masayuki Fukumura	4001-0003	8941

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EXAMINER	
KATCHEVES, KONSTANTINA T	
ART UNIT	**PAPER NUMBER**
1636	

DATE MAILED: 01/07/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Notice of Abandonment	Application No.	Applicant(s)	
	09/720,979	FUKUMURA ET AL.	
	Examiner	Art Unit	
	Konstantina Katcheves	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

This application is abandoned in view of:

1. ☒ Applicant's failure to timely file a proper reply to the Office letter mailed on *08 June 2004*.
   (a) ☐ A reply was received on _____ (with a Certificate of Mailing or Transmission dated _____), which is after the expiration of the period for reply (including a total extension of time of _____ month(s)) which expired on _____.
   (b) ☐ A proposed reply was received on _____, but it does not constitute a proper reply under 37 CFR 1.113 (a) to the final rejection.
   (A proper reply under 37 CFR 1.113 to a final rejection consists only of: (1) a timely filed amendment which places the application in condition for allowance; (2) a timely filed Notice of Appeal (with appeal fee); or (3) a timely filed Request for Continued Examination (RCE) in compliance with 37 CFR 1.114).
   (c) ☐ A reply was received on _____ but it does not constitute a proper reply, or a bona fide attempt at a proper reply, to the non-final rejection. See 37 CFR 1.85(a) and 1.111. (See explanation in box 7 below).
   (d) ☒ No reply has been received.

2. ☐ Applicant's failure to timely pay the required issue fee and publication fee, if applicable, within the statutory period of three months from the mailing date of the Notice of Allowance (PTOL-85).
   (a) ☐ The issue fee and publication fee, if applicable, was received on _____ (with a Certificate of Mailing or Transmission dated _____), which is after the expiration of the statutory period for payment of the issue fee (and publication fee) set in the Notice of Allowance (PTOL-85).
   (b) ☐ The submitted fee of $_____ is insufficient. A balance of $_____ is due.
   The issue fee required by 37 CFR 1.18 is $_____. The publication fee, if required by 37 CFR 1.18(d), is $_____.
   (c) ☐ The issue fee and publication fee, if applicable, has not been received.

3. ☐ Applicant's failure to timely file corrected drawings as required by, and within the three-month period set in, the Notice of Allowability (PTO-37).
   (a) ☐ Proposed corrected drawings were received on _____ (with a Certificate of Mailing or Transmission dated _____), which is after the expiration of the period for reply.
   (b) ☐ No corrected drawings have been received.

4. ☐ The letter of express abandonment which is signed by the attorney or agent of record, the assignee of the entire interest, or all of the applicants.

5. ☐ The letter of express abandonment which is signed by an attorney or agent (acting in a representative capacity under 37 CFR 1.34(a)) upon the filing of a continuing application.

6. ☐ The decision by the Board of Patent Appeals and Interference rendered on _____ and because the period for seeking court review of the decision has expired and there are no allowed claims.

7. ☐ The reason(s) below:

The abandonment of the present application was confirmed by Mark Shanks on 27 December 2004.

JAMES KETTER
PRIMARY EXAMINER

Petitions to revive under 37 CFR 1.137(a) or (b), or requests to withdraw the holding of abandonment under 37 CFR 1.181, should be promptly filed to minimize any negative effects on patent term.

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Taylor & Francis

Therapeutic gene transfer to the nervous system using viral vectors

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The past few years have been marked by substantial progress in preclinical studies of therapeutic gene transfer for neurologic disease using viral-based vectors. In this article, the authors review the data regarding (1) treatment of focal neuronal degeneration, exemplified by Parkinson disease, ischemia, and trauma models; (2) treatment of global neurologic dysfunction, exemplified by the mucopolysaccharidoses and other storage diseases; (3) peripheral nervous system diseases including motor neuron disease and sensory neuropathies; and (4) the use of vectors expressing neurotransmitters to modulate functional neural activity in the treatment of pain. The results suggest that a number of different viral vectors may be appropriate for gene transfer to the central nervous system for specific disease processes, and that for the peripheral nervous system herpes simplex virus–based vectors appear to have special utility. The results of the first human gene therapy trials for neurologic disease, which are just now beginning, will be crucial in defining the next step in the development of this therapy. *Journal of NeuroVirology* **(2003) 9, 165–172.**

Keywords: gene therapy; pain; Parkinson disease; mucopolysaccharidosis; neuropathy

Introduction

It has been 30 years since gene therapy was first formally proposed as a treatment for genetically determined inherited disorders (Friedmann and Roblin, 1972). Despite the setback caused by the well-publicized death of one patient in a gene therapy trial in 1999 (Carmen, 2001), the first successful human gene therapy, for X-linked severe combined immunodeficiency in children, has been reported (Cavazzana-Calvo *et al*, 2000). In recent years, several proposed human gene therapy protocols for neurologic disease have been reviewed by the recombinant DNA advisory committee (RAC) of the National Institutes of Health (NIH) and a number of these are now in clinical trial. It is thus an apt time to consider the progress of gene therapy for neurologic disease, and the prospects for future advances in the field.

There are several reasons that therapeutic gene transfer or "gene therapy" might be particularly appropriate for treating conditions affecting the nervous system. More unique RNA sequences are expressed in brain than in any other tissue and a large proportion of the identified genetic diseases display a neurologic component to the phenotype. The blood-brain barrier limits the penetration of systemically administered macromolecules into brain, and macromolecules injected directly into the ventricles penetrate only a short distance into brain parenchyma. In many cases, the regional specialization of brain function dictates that a therapeutic intervention may be best achieved by the local expression of a transgene product such as a neurotrophic or antiapoptotic factor. In addition, the widespread and redundant use of a limited repertoire of neurotransmitters and receptors in diverse pathways in the nervous system means that the local production of neurotransmitters achieved by therapeutic gene transfer may be used to achieve desired outcomes while avoiding unwanted adverse side effects that would result from activation of the same receptors in other pathways by a

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systemically administered drug. Nonviral means of gene transfer, such as liposomes, have generally proven ineffective for gene transfer to the nervous system. On the other hand, a number viral-based vectors, including those based on viruses such as lentivirus (LV) or herpes simplex virus (HSV) that naturally infect the nervous system, or developed from viruses like adenovirus (Ad) or adenoassociated virus (AAV) that are not naturally neurotropic, have proven effective in different model systems.

In this review, we summarize the published data to date regarding therapeutic gene transfer using viral vectors in animal models of neurologic disease, and describe several human trials of therapeutic gene transfer for neurologic disease that have been approved by regulatory agencies, some of which are now enrolling patients. The review is focused on preclinical studies in animal models of neurologic disease, and their translation to human therapy. Progress in four different specific applications relevant to neurologic disease will be reviewed: (1) treatment of focal neuronal degeneration, exemplified by Parkinson disease, ischemia, and trauma models; (2) treatment of global neurologic dysfunction, exemplified by the mucopolysaccharidoses and other storage diseases; (3) peripheral nervous system diseases including motor neuron disease and sensory neuropathies; and (4) the use of vectors expressing neurotransmitters to modulate functional neural activity in the treatment of pain. The use of gene transfer to modify cells that are subsuquently implanted into brain or spinal cord (Blesch *et al*, 2002; Tuszynski, 1997), and the reports regarding the use of gene transfer in the treatment of glioblastoma, either by direct cell killing, immunologic effects, or suicide gene therapy (Andratschke *et al*, 2001; Markert *et al*, 2001), will not be considered in this review. The basic biology of the principal vectors that are used in these applications has been reviewed elsewhere (Kennedy, 1997).

Treatment of focal neurodegeneration: Parkinson disease, stroke, and trauma

Focal neurodegeneration would appear to be an ideal target for therapeutic gene transfer. Despite the fact that the pathogenic mechanisms underlying progressive cell death in neurodegenerative disease are incompletely understood, several peptides that act either as trophic factors or to interrupt the apoptotic cascade intracellularly have been identified. It is unlikely that such potent substances delivered either systemically or intrathecally would not cause serious adverse effects (Apfel, 2001). Because gene transfer offers the possibility of local production of such factors to prevent neurodegeneration, a number of investigators have focused on this possibility. Idiopathic Parkinson disease (PD), a condition characterized by degeneration of dopaminergic (DA) neurons in the substantia nigra (SN), has the advantage of a very restricted anatomic target (the SN) and well-characterized animal models. The first studies of gene transfer in PD, employing the model of 6-hydroxydopamine (6-OHDA)–induced degeneration of DA cells in the SN, demonstrated that intrastriatal injection of an Ad vector expressing the glial cell–derived neurotrophic factor (GDNF) prevented the degeneration of DA neurons, resulting in both histologic and behavioral correction of the disease phenotype (Bilang-Bleuel *et al*, 1997). Subsequent studies have confirmed these results using AAV-based vectors (Mandel *et al*, 1997, 1999), other Ad vectors (Choi-Lundberg *et al*, 1998; Connor *et al*, 1999; Bjorklund *et al*, 2000), replication-defective HSV vectors (Yamada *et al*, 1999), and LV vectors (Bensadoun *et al*, 2000). Protection of DA neurons from 6-OHDA toxicity *in vivo* has also been reported in experiments in which the antiapoptotic peptide Bcl-2 was expressed using an HSV vector in the rat (Yamada *et al*, 1999). Both the LV (Kordower *et al*, 2000) and AAV (Bjorklund *et al*, 2000) experiments have been shown to protect DA neurons in primates. No human trials to prevent cell death in PD based on the preclinical data generated have been proposed to date.

An alternate gene transfer approach to the treatment of PD utilizes gene transfer designed to enhance neurotransmitter production in the striatal circuitry damaged in PD. The most obvious candidate is tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine synthesis. Injection of an AAV vector expressing TH into striatum was first demonstrated to reverse one behavioral abnormality in the 6-OHDA model of PD (Kaplitt *et al*, 1994), and similar results were obtained with an HSV-based amplicon vector expressing TH (During *et al*, 1994). However the size of the human striatum, the likely requirement that dopamine production will need be closely regulated to avoid adverse effects, combined with the complexity and variability of PD symptomatology, make this type of therapy problematic. Modulation of neurotransmitter effect can be achieved by enhancing prodrug conversion. It has been demonstrated that transfer of the gene coding for the aromatic acid decarboxylase (AADC) enhances the conversion of DOPA, administered systemically, to dopamine (Sanchez-Pernaute *et al*, 2001). The first human PD gene transfer trial, on the other hand, has proposed to transfer the gene coding for glutamic acid decarboxylase (GAD) in order to increase γ-aminobutyric acid (GABA) expression in the extrapyramidal pathway (During *et al*, 2001). In the phase I trial that has been proposed, the vector will be inoculated along with the placement of a deep brain stimulator into the subthalamic nucleus.

Therapeutic results of focal gene transfer has been demonstrated in models of ischemic brain injury in rodents using a variety of vectors. Expression of

interleukin-1 receptor antagonist from an Ad vector (Betz *et al*, 1995), Bcl-2 from an HSV amplicon vector (Lawrence *et al*, 1997) or from an AAV vector (Shimazaki *et al*, 2000), GDNF from an AAV vector (Tsai *et al*, 2000), and heat shock protein (HSP) 72 from an HSV amplicon (Hoehn *et al*, 2001) have all been shown to attenuate the amount of cell loss in a variety of models of transient and permanent ischemia. Although these "proof-of-principle" studies, demonstrate a biological activity of gene transfer, not all of the studies have been correlated with behavioral outcomes that would be required to support the clinical use, and in all of these studies, the vectors have been injected prior to the ischemic insult, which would severely limit the clinical situations for which such gene transfer would be applicable. Similar results have also been demonstrated in models of nervous system trauma. Injection of HSV vectors expressing Bcl-2 or GDNF up to 30 min after spinal root avulsion improves motor neuron survival and preserves expression of choline acetyltransferase in lesioned motor neurons (Natsume *et al*, 2002; Yamada *et al*, 2001). Intraspinal injection of a plasmid encoding Bcl-2 complexed in a lipsome immediately following spinal cord section has been demonstrated to protect neurons of Clark's nucleus and the red nucleus from injury-induced degeneration (Shibata *et al*, 2000; Takahashi *et al*, 1999), and intraspinal application of vascular endothelial growth factor (VEGF) using an Ad vector appears to ameliorate the effect of a corticospinal tract injury in rodents (Facchiano *et al*, 2002). Injection of an Ad vector expressing neurotrophin-3 (NT-3) into spinal cord after dorsal root injury enhanced the regeneration of a subpopulation of dorsal root axons (probably myelinated A fibers), into and through the CNS environment (Zhang *et al*, 1998). Injection of Ad vectors expressing fibroblast growth factor-2 (FGF2) or nerve growth factor (NGF) 16 days after dorsal root injury induced robust axonal regeneration into normal as well as ectopic locations within the dorsal spinal cord, resulting in near-normal recovery of thermal sensory function (Romero *et al*, 2001). Fewer unwanted adverse effects were seen with FGF2 than with NGF.

Correction of global brain disease: Mucopolysaccharidoses and other storage diseases

Gene transfer has also been applied to the treatment of diseases that affect the central nervous system globally. In these cases, the aim of gene transfer is a diffuse distribution of the corrective gene product throughout the nervous system. It was originally demonstrated that administration of a recombinant Ad vector expressing beta-glucuronidase directly into the lateral ventricles of mutant mice increased the beta-glucuronidase activity in crude brain homogenates to 30% of heterozygote activity. Histochemical demonstration of beta-glucuronidase activity in brain revealed that the enzymatic activity was found principally in ependymal cells and choroids plexus (Ohashi *et al*, 1997). An adenovirus vector expressing aspartylglucosaminidase (AGA) injected intraventricularly into the brain mice with aspartylglucosaminuria (AGU) resulted in AGA expression in the ependymal cells lining the ventricles and diffusion of AGA into the neighboring neurons. One month after administration of the wild-type Ad-AGA, a total correction of lysosomal storage in the liver and a partial correction in brain tissue surrounding the ventricles was observed (Peltola *et al*, 1998). Similar results have been demonstrated in the mucopolysaccharidosis (MPS) VII mouse injected with an Ad vector expressing beta-glucuronidase, with the distribution of enzyme activity and phenotypic correction increased by mannitol-induced disruption of the brain–cerebrospinal fluid (CSF) barrier (Ghodsi *et al*, 1999). Using the same models, others have shown that AAV vectors expressing beta-glucuronidase injected directly into brain parenchyma can result in phenotypic correction (Sferra *et al*, 2000; Skorupa *et al*, 1999). Wolfe and coworkers reported that the AAV vector not only produced the normal enzyme from infected cells at the injection sites, but that the secreted enzyme was also disseminated along most of the neuraxis, resulting in widespread reversal of the hallmark pathology. The extensive area of correction surrounding the transduction sites suggested that a limited number of appropriately spaced sites of gene transfer may provide overlapping spheres of enzyme diffusion to cover a large volume of brain tissue (Bosch *et al*, 2000a, 2000b; Skorupa *et al*, 1999). AAV-mediated correction has been reported to improve cognitive function in the murine model of MPS VII as measured by the Morris water maze test (Frisella *et al*, 2001). More recently, Davidson and coworkers have demonstrated that injection of a feline immunodeficiency virus (FIV)-based vector expressing beta-glucuronidase into striatum unilaterally resulted in bihemispheric correction of the characteristic cellular pathology and that treatment of beta-glucuronidase–deficient mice with established impairments in spatial learning and memory resulted in a dramatic recovery of behavioral function (Brooks *et al*, 2002).

In the mouse model of MPS IIIB resulting from a defect in alpha-*N*-acetylglucosaminidase (NaGlu), an NaGlu-expressing AAV vector injected into brain resulted in 6 months of expression of recombinant NaGlu (rNaGlu) in multiple brain regions of adult MPS IIIB mice. The vector transduced an area of 400 to 500 microns surrounding the infusion sites, but after 6 months, the correction of glycose aminoglycan storage involved neurons of a much larger area (Fu *et al*, 2002). In a mouse model of metachromatic leucodystrophy, Naldini and coworkers demonstrated that a lentiviral vector encoding a functional

arylsulfatase A (ARSA) gene injected into the brain of adult mice with germ-line inactivation of the mouse gene encoding ARSA resulted in sustained expression of active enzyme throughout a large portion of the brain, with long-term protection from development of neuropathology and hippocampal-related learning impairments (Consiglio *et al*, 2001).

Correction of phenotypic deficits in both histology and behavior in MPS mice using gene transfer has been impressive, and the reversal of established deficits (Brooks *et al*, 2002) represents an important clinical feature in consideration of the development of a practical treatment. Several features of this model should be kept in mind. The relevant gene product is taken up by cells throughout the brain by binding to mannose-6-phosphate receptors. Thus, global correction of these diseases can be achieved by transduction of a fraction of cells within the brain as long as the gene product released from the cells is adequately distributed through the brain. In other models using enzyme replacement, it has been noted that replacement of as little as 10% of the normal enzyme activity may be sufficient to correct the phenotype. Regarding the application to human disease, issues of volume of distribution need to be explored. Even though correction of an animal model has not yet been demonstrated, a human trial of gene transfer to treat Canavan disease using liposomes to transfer aspartoacylase has been reported (Leone *et al*, 2000), and the same group has now begun a similar study in children using an AAV vector.

Diseases of the peripheral nervous system: Polyneuropathy and motor neuron disease

The peripheral nervous system presents a number of challenges that are distinct from the central nervous system, but the underlying rationale for the use of gene therapy is similar. Studies with recombinant peptides have demonstrated that a number of neurotrophic factors, including NGF, NT-3, insulin-like growth factor (IGF), and vascular epithelial growth factor (VEGF) can prevent the degeneration of peripheral sensory axons that results in polyneuropathy (Apfel, 1999). But these potent short-lived peptides cannot be administered to patients in the same doses that are effective in the animal models because of unwanted adverse systemic effects (Apfel, 2002). One approach to this problem is to selectively transduce dorsal root ganglion neurons to express a neurotrophic factor in order to achieve local (autocrine or paracrine) protective effect while avoiding systemic side effects. In this regard, HSV-based vectors are particularly well suited because of the natural tropism of the wild-type virus that affords efficient uptake into dorsal root ganglion (DRG) neurons from peripheral inoculation of the vector (Mata *et al*, 2001).

Using transduction of DRG neurons by peripheral inoculation of an HSV vector, we have demonstrated a protective effect against the development of neuropathy in three different models of polyneuropathy. Selective large fiber nerve degeneration caused by overdose of pyridoxine (PDX) can be prevented by subcutaneous inoculation of an HSV-based vector containing the coding sequence for NT-3, measured by the amplitude and conduction velocity of the evoked sensory response, as well as preservation of H-wave amplitude (Chattopadhyay *et al*, 2002). Treated animals show preservation of a population of large myelinated fibers that otherwise degenerate in this condition, and the preservation of electrophysiologic and histologic parameters is reflected in behavioral testing of treated animals (Chattopadhyay *et al*, 2002). Inoculation of an HSV-based vector expressing NGF under the control of the human cytomgalovirus promoter (HCMV) prior to the start of PDX intoxication provides a similar protective effect (Chattopadhyay *et al*, 2003). Similarly, injection of an replication-incompetent HSV vector expressing NGF under the control of the HCMV promoter 2 weeks after the induction of diabetes (by injection of streptozotocin) prevents the development of neuropathy, measured by reduction in evoked sensory nerve amplitude, and also increases expression of neuropeptides in the DRG (Goss *et al*, 2002a). Similar results have been obtained in a model of drug-induced sensory neuropathy resulting from administration of cisplatin (Chattopadhyay *et al*, personal communication). Iatrogenic neuropathies caused by chemotherapy for cancer are models that may be tested in human disease. A similar protective effect has been observed by transfer of VEGF using a plasmid injected into muscle in models of ischemic and diabetic neuropathy (Schratzberger *et al*, 2000, 2001), although one must assume that the protective effect in those models results from circulating levels of VEGF achieved by muscle transduction and thus may not avoid the potential for systemic side effects.

Motor neuron disease is a serious and fatal affliction without currently effective treatment. Like polyneuropathies, administration of trophic factors appears to slow the progression of the disease in rodent models, but a human trial of ciliary neuronotrophic factor (CNTF) in motor neuron disease had to be abandoned because of the cytokine-like side effects of the systemically administered trophic factor (Apfel, 2002). An AAV-based vector expressing GDNF has been demonstrated to protect a motor neuron-like cell line from apoptotic cell death *in vitro* (Keir *et al*, 2001). After intramuscular injection of the NT-3 adenoviral vector, pmn mice (a model of motor neuron disease) showed a 50% increase in life span, reduced loss of motor axons, and improved neuromuscular function as assessed by electromyography. These results were further improved by coinjecting an adenoviral vector coding for CNTF (Haase *et al*, 1997). Administration of an adenoviral vector expressing cardiotrophin 1 (CT-1) to newborn pmn

mice led to sustained CT-1 expression in the injected muscles and bloodstream, prolonged survival of animals, and improved motor functions. CT-1–treated mice showed a significantly reduced degeneration of facial motor neurons and phrenic nerve myelinated axons. The terminal innervation of skeletal muscle, grossly disturbed in untreated pmn mice, was almost completely preserved in CT-1–treated pmn mice (Bordet *et al*, 1999). This approach relies on systemic release from injected muscle, and thus may not avoid the problems of systemic administration. Achieving adequate systemic levels from muscle transduction in larger animals may prove difficult. To date, no vectors have been created from viruses that would naturally target motor neurons in a manner similar to the targeting of DRG neurons by HSV-based vectors, and efforts to construct vectors that would target to motor neurons have to date been unsuccessful.

Gene transfer for the treatment of pain

In a manner analagous to the correction of PD by using gene transfer to achieve focal neurotransmitter release (transduction with a TH vector to produce DA, transduction with a GAD-expressing vector to produce GABA), several studies have demonstrated that gene transfer may be used to provide an analgesic effect in the treatment of pain. Opiate drugs are exceptionally potent analgesic agents, but the action of these drugs on central and peripheral opioid receptors resulting in nausea, sedation, respiratory suppression, and constipation or urinary retention, respectively, limit the dose that may be used. Continued use of opiate drugs in chronic pain leads to tolerance, and addiction is also a problem. Several different gene transfer approaches have been taken to the treatment of pain.

Iadarola and coworkers demonstrated that a recombinant Ad encoding a secreted form beta-endorphin injected intrathecally into lumbar CSF transduced meningeal cells, and that beta-endorphin secretion attenuated inflammatory hyperalgesia, without affecting basal nociceptive response (Finegold *et al*, 1999). HSV-mediated gene transfer to deliver and express opioid peptides to be released from primary afferent terminals may be used to alter the physiology of postsynaptic neurons, affecting nociceptive transmission in the spinal dorsal horn. An HSV vector containing the human proenkephalin gene injected subcutaneously in the foot produces an antihyperalgesic effect in rodents (Wilson *et al*, 1999), and a 50% reduction in the spontaneous pain behavior during the delayed phase of the formalin test of inflammatory pain (Goss *et al*, 2001). The naltrexone-reversible analgesic effect in inflammatory pain is maximal 1 week after vector inoculation, and can be reestablished by reinoculation of the vector after the initial effect has waned (Goss *et al*, 2001). In the spinal nerve ligation (SNL) model of neuropathic pain, injection of the vector 1 week after SNL produced a naloxone-reversible antiallodynic effect that was continuous, persisted for several weeks, and could also be reestablished by reinoculation of the vector after the original effect had waned. In the neuropathic pain model, vector-mediated enkephalin expression enhances the effect of morphine, reducing the ED_{50} of morphine from 1.8 mg/kg to 0.15 mg/kg, and the vector continues to provide an antiallodynic effect in the face of tolerance to morphine induced by repeated injection of the drug (Hao, personal communication). A similar analgesic effect for HSV-mediated expression of proenkephalin has been demonstrated in a model of polyarthritis (Braz *et al*, 2001), and in a rodent model of pain caused by cancer in bone (Goss *et al*, 2002b). We have presented a proposal for a phase I human trial of the proenkephalin-expressing vector in the treatment of pain resulting from cancer metastatic to bone to the RAC in June, 2002.

Summary and conclusion

In the last 5 years, substantial progress has been made in moving gene transfer for neurologic disease from a hypothetical possibility to a real treatment. The data considered in this review suggest that a number of different vectors (Ad, AAV, LV, HSV) may be used for focal gene transfer to the central nervous system. The choice among these vectors will ultimately be decided by the results of the human trials, and practical aspects of manufacturing. For global distribution within the brain, it would appear that the smaller vectors (AAV and LV) may be advantageous, but the problem of delivering a gene product to the entire human brain from focal injections would appear to be daunting. For peripheral sensory nervous system applications, including the prevention of neuropathy and the treatment of pain, HSV, because of its natural tropism to sensory neurons, would appear to be the vector of choice. No vectors with similar tropism to motor neurons have yet been demonstrated.

As outlined in this review, potent therapeutic effects of gene transfer have now been demonstrated in several relevant models of different neurologic diseases. A human trial of gene transfer for Canavan disease (using liposomes and AAV vectors) is underway, and trials for Parkinson disease (using an AAV vector expressing GAD) and for the treatment of pain (using an HSV vector expressing proenkephalin) have passed through the RAC to the Food and Drug Administration (FDA). Although novel vectors that may extend the range of therapeutic options continue to be developed, the observations from the first human trials will be crucial in defining the next step in the development of this therapy.

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